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Alterations to Embryonic Serotonin Change Aggression and Fearfulness

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Prenatal stress can alter the serotonin (5-HT) system in the developing and adult brain and lead to mood and behavioral disorders in children and adults. The chicken provides a unique animal model to study the effects of embryonic stressors on childhood and adolescent behavior. Manipulations to the egg can be made in the absence of confounding maternal effects from treatment. Eggs were injected with 50 μL of excess 5-HT (10 μg/egg), 8-OH-DPAT (a 5-HT1A receptor agonist; 16 μg/egg), or saline on day 0 prior to the 21 days incubation. Injections were performed at 0.5 cm below the shell. Behavior was analyzed at 9 weeks of age and again at the onset of sexual maturity (18 weeks). Hens treated with excess embryonic 5-HT exhibited significantly less aggressive behaviors at 9 weeks of age compared to both 5-HT1A agonist treated and saline hens ($P < 0.05$), and at 18 weeks of age compared to saline control birds only ($P < 0.05$). Excess embryonic 5-HT also increased fearfulness response ($P < 0.05$) as tested by duration of tonic immobility. Increased degree of fluctuating asymmetry at 18 weeks in 5-HT-treated birds ($P < 0.05$) suggests that excess 5-HT in early embryonic stages may create a developmental instability, causing phenotypic variations. These results showed that modification of the serotonergic system during early embryonic development alters its functions in mediating aggressive and fearful or anxious behaviors. Prenatal modification of the serotonergic system has long lived implications on both physiology and behavior, especially aggressive and fearful behaviors. Aggr. Behav. 39:91–98, 2013. Published 2013 Wiley Periodicals, Inc.†

Keywords: serotonin, aggression, fearfullness, avian, embryonic development

INTRODUCTION

Investigation of alterations to prenatal neural development often focuses on prenatal stress (specifically glucocorticoid alterations) or the effects of prenatal exposure to addictive substances such as nicotine, alcohol, and cocaine with long-term effects on behavioral exhibition and physiological homeostasis (D'Angiulli, Grunau, Maggi, & Herdman, 2006; Liu & Wuerker, 2005; Levitt, 1998; Oberlander et al., 2010; Rice & Barone, 2000). Currently in America, 18.1% of adults (or 40 million adults) suffer from an anxiety disorder, 6.7% have been diagnosed with major depressive disorder, and 26.2% of the population suffer from some type of mental disorder (NIMH, 2011). Often, these disorders are treated with the use of SSRI (selective serotonin reuptake inhibitor) medications (Oberlander et al., 2010), as further indicated in animal models (Hofmann et al., 2001; Meerlo et al., 2001). However, the long-term effects of prenatal exposure to altered serotonin secretion on behavioral and neural development are only recently being investigated.

Serotonin (5-HT) plays a vital role in both embryonic neural development and aggressive behavioral regulation. Previous studies have shown the inhibition of 5-HT transport during early postnatal development can cause abnormal emotional behavior in adult mice (Ansorge, Morelli, & Gingrich, 2008). In addition, changes in monoamine levels including 5-HT can retard embryogenesis in animals such as rats (Hansson & Thorlin, 1999; Powrozek & Zhou, 2005) and snails (Filla, Hiripi, & Elekes, 2004, 2009).

Fetal 5-HT receptors have been shown to be functional and play a vital role in neural development in the brain of rodents (Hellendall, Schambra, Liu, & Lauder, 1993; Whitaker-Azmitia, Lauder, Shemmer, & Azmitia, 1987) and humans (Pascual, del Arco, Romon, delOlmo, & Pazos, 1996) during the embryonic phase. Mice exposed prenatally to SSRIs (selective 5-HT reuptake inhibitors, functionally as 5-HT agonists) exhibited increased anxiety

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and aggression related behaviors (Coleman, Christensen, Gonzalez, & Rayburn, 1999). Human studies have shown that children who were exposed prenatally to both SSRI medications and a depressed maternal mood exhibited increased internalizing behaviors (Oberlander et al., 2010). Internalizing behavioral problems (including depression, anxiety and social abnormalities) have been seen in children exposed to excess 5-HT prenatally.

The use of SSRI medications has been found to desensitize 5-HT1A receptors as well as to down regulate serotonin transporters [Parsey et al., 2005]. 5-HT1A receptors play a vital role in the regulation of aggressive behaviors [Nelson and Chiavegatto, 2001]. A study by Linmarie et al. [1990] evidenced a decrease in neurite branching during development following excitation of the 5-HT1A receptors with 8-OH-DPAT (a 5-HT1A receptor agonist). Mice from high and low aggressive strains given buspirone (another 5-HT1A receptor agonist) on 2–6 days postnatally developed greater and less aggressiveness, respectively [Markina et al., 2006]. Both of these agonists have been shown to cause serotonin syndrome in sufficient doses; however, buspirone and not 8-OH-DPAT has also been shown to reduce overall activity [Ferreira et al., 2000], for that reason 8-OH-DPAT was chosen for the present study.

The chicken has been suggested to provide an excellent model for prenatal stress [Lay and Wilson, 2002]. Embryonic development is similar to that seen in mammalian species; however, maternal feedback is eliminated. In the present study, we adapt this model of prenatal stress to investigate the effects of altered serotonin on later aggressive and fear-related behaviors.

A previous study by Ahmad and Zemenhof [1978] reported that chick embryos subjected to an injection of excess 5-HT at day 7 of incubation were found to have increased 5-HT concentrations at day 10 as well as overall greater cerebral and optic lobe weights. However, little work has been done in the long-term neurophysiological and behavioral effects of excess 5-HT delivered early in embryonic development. In this study, we tested the hypotheses that (i) a single dose of 5-HT or 5-HT1A agonist (using 8-OH-DPAT) delivered immediately prior to incubation will have lasting effects on post hatch neurophysiology and behaviors in birds up to 18 weeks of age, and (ii) the effects of early 5-HT on behavior and neurophysiology work, at least in part, through the 5-HT1A receptor function.

METHOD

Experimental Design

Two hundred and forty fertilized White Leghorn eggs were injected with 50 μl of one of the following treatments: serotonin (10 μg/egg), 8-OH-DPAT (a 5-HT1A receptor agonist; 16 μg/egg) or saline controls on day 0 prior to a period of 21 days incubation (n = 80 per treatment). At 18 day of incubation, eggs were candled and viable eggs were transferred to separated hatching baskets where they remained until hatch. Upon hatching, birds were transferred to 2 ft square plexiglass floor pens and were housed three birds per pen within the same treatment. Birds were provided ad libitum access to water and standard feed appropriate for their stage of development and were kept at a constant 22 hr light/2 hr dark photoperiod for the first week, gradually reducing the light duration to 16 hr light/8 hr dark at about 18 weeks.

Behavior

At 9 and 18 weeks of age birds were placed in plexiglass cages with one bird from each treatment (saline, 5-HT- and 8-OH-DPAT-treated eggs, i.e. three birds/cage; $N = 10$/treatment). A different cohort of birds was used for each of the above-listed time periods. Each cage provided 192 in^2 per bird. Birds' behaviors were recorded using a 16-channel digital video recording system for 1 hr and analyzed for aggressive behaviors given including frequency of aggressive pecks, kicks, and threats. The definitions for each behavior are given below.

- *Aggressive pecking*: forceful downward pecks directed at the head or neck of other birds.
- *Threat*: one bird standing with its neck erect and hackle feathers raised (may only be slightly) in front of another bird.
- *Kicking*: one bird forcefully extending one or both legs such that the foot strikes another bird.

Tonic immobility (TI) test is routinely used as a fear test. It was performed in a V-shaped cradle as described by Jones [1986] at 18 weeks of age. Birds were given up to five attempts to induce TI and remained in the cradle until the bird righted itself or until the time limit of 10 min was reached. Number of inductions and duration were both recorded. TI was performed by one observer and in a separate room from housed birds in order to reduce variability and eliminate potential distractions from birds not in testing.

Measure for Fluctuating Asymmetry Analysis

Shank length and width of both sides of feet were measured using dial calipers. Fluctuating asymmetry [FA, Dennis et al., 2008] was assessed as the absolute difference in shank width plus absolute difference in shank length, using the equation below:

$$\mathrm{FA(length)} = |(\text{right shank length}) - (\text{left shank length})|$$

$$\mathrm{FA(width)}\ |(\text{right shank width}) - (\text{left shank width})|$$

Physiology

Five birds from each treatment were sampled at 1 day, 15 days, 35 days, 9 weeks, and 18 weeks of age, and were euthanized by cervical dislocation. Immediately following euthanasia the brains were removed, and the brain region containing the dorsal raphe nucleus and the hypothalamus were dissected using a stereotaxic atlas [Puelles et al., 2007] and maintained at −80°C until it was prepared for assay by high performance liquid chromatography (HPLC). Central 5-HT, EP (epinephrine), NE (nonepinephrine), DA (dopamine), and their metabolites, 5-hydroxyindoleacetic acid (5HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were measured in duplicate from the brain samples as previously described by Cheng and Fahey [2009]. Briefly, samples were acidified in duplicate using a 10:1 dilution of 0.2 M perchloric acid and freshly prepared 3% ascorbic acid. Samples were centrifuged and diluted with 50% mobile phase (MD-TM ESA Mobile Phase; ESA, Inc.) before being filtered through a 0.2 μg nylon filter. The mobile phase flow rate was 1.2 mL/min, and the concentration of neurotransmitters and metabolites were calculated from a reference curve made using standards. Concentrations were obtained as nanograms per milliliter.

Real-Time PCR

RNA extraction. Extraction of total RNA was performed on the right half of the collected brain tissue. Extraction and homogenization were performed using RNEasy Mini kits and Quiashredders (Qiagen, Valencia, CA), respectively.

cDNA synthesis. Reverse transcription was performed as described in Williams et al. [2008]. In short, RNA samples were diluted with RNase free water based on RNA concentration. Master mix was made from TaqMan Reverse Transcription Reagent Pack (Applied Biosystems, Foster City, CA) and added to dilute RNA samples. Samples were then placed in a Hybrid PCR Express thermo cycler (Midwest Scientific, St. Louis, MO). The resulting cDNA was stored at −80°C until further analysis.

Primers and probes. Primer Express software was used to develop all primers and probes used in real-time PCR. Primer and probe synthesis was performed by Applied Biosystems for the following genes 5-HT1A receptor (forward primer: GGCCGCCGTGCTCAT; reverse primer: ATGGGCGGGATGGATATCA; and probe: CCTGACCTGGCTCAT), 5-HT1B receptor (forward primer: ACGTCAATACATACTGCCAGCAA; reverse primer: CTCTGCCAGAGTCATTACACTGAAA; and probe: CTGCTCAGCTCTGC), and the reference gene actin (forward primer: CCCATTGAACACGGCATCA; reverse primer: GGTGTGGTGCCAGATCTTCTC; and probe: CACAAACTGGGACGACAT).

Quantification of expression of genes of interest. Real-time PCR was conducted on the cDNA product using Applied Biosystems procedures [Williams et al., 2008].

Statistics

Behavior and physiological data were analyzed using PROC MIXED of SAS 9.0 software (Cary, NC), main effects included age and embryonic treatment. Interactions between main effects were also considered. Log transformation was used in analysis of variance (ANOVA) of catecholamines and amino acid data. Least square means (LSMeans) and standard error of the mean (SEM) were reported for all groups. Contrasts were used to determine significance of means using the Bonferroni adjustments when required to maintain an experimental alpha of 0.05 (0.10 was considered a trend). Descriptive statistics for each ANOVA, including denominator degrees of freedom, numerator degrees of freedom, F statistics, and P values are provided (Table I).

RESULTS

Behavior

Aggressive threats were decreased in birds from 5-HT-treated eggs compared with controls at both 9 (0.01 ± 1.38 and 4.8 ± 1.38 threats per 30 min, respectively) and 18 weeks of age (6.9 ± 4.94 and 19.7 ± 4.94 threats per 30 min, respectively; $P < 0.05$; Fig. 1). However, birds from eggs treated with 5-HT1A agonist did not differ from controls in number of threats (5.9 ± 1.38 and 14.6 ± 4.94 threats per 30 min at 9 and 18 weeks, respectively; $P > 0.05$; Fig. 1). Fear response as measured by TI duration was elevated in birds from 5-HT-treated eggs compared with controls (580 ± 59 and 392 ± 59 sec, respectively; $P < 0.05$; Fig. 2) but not in birds from 8-OH-DPAT-treated eggs (462 ± 59 sec; $P > 0.05$; Fig. 2).

Physiology

Serotonin concentration, compare to controls, was elevated in birds from 5-HT-treated eggs at 18 weeks ($P < 0.05$) and tended to be elevated at day 1 post hatch ($P = 0.1$; Table II). Among the treatments, 8-OH-DPAT birds had highest central concentrations of 5-HIAA at 1 day of age while the highest 5-HIAA concentrations were found in the saline birds at 9 weeks of age ($P < 0.05$; Table II). Overall serotonin turnover was elevated in 8-OH-DPAT birds and reduced in 5-HT birds

TABLE I. Statistical Values From ANOVAs Including Numerator Degrees of Freedom (nDF), Denominator Degrees of Freedom (dDF), *F*-Statistics (*F*) and Probability Values (*P*)

	nDF	dDF	*F*	*P*<
Aggressive behavior (9 week)				
Threats	2	18	3.93	0.0424
Pecks	2	18	1.36	0.2818
Aggressive behavior (18 week)				
Threats	2	18	4.00	0.0366
Pecks	2	18	0.25	0.7832
Neurotransmitter concentration (treatment * day)				
Serotonin	8	61	2.44	0.0233
5-HIAA	8	61	2.17	0.0423
Serotonin turnover	8	61	3.88	0.0009
Dopamine	8	61	2.21	0.0388
DOPAC	8	61	2.48	0.0213
HVA	8	61	2.20	0.0396
Dopamine turnover	8	61	2.13	0.0462
Epinephrine	8	61	0.63	0.7494
Norepinephrine	8	61	0.88	0.5385
Gene expression (treatment * day)				
5-HT1A expression	8	61	2.31	0.0311
5-HT1B expression	8	61	0.53	0.8322
Gene expression (by day only)				
5-HT1B expression	4	61	2.56	0.0474
Fluctuating asymmetry at week 18				
Fluctuating asymmetry (width)	2	15	6.75	0.0081
Fluctuating asymmetry	2	15	5.10	0.0204

at 1 day of age ($P < 0.05$; Table II). At 9 weeks of age, compared with control (saline) birds, both treated groups remained at low levels of serotonin turnover ($P < 0.05$; Table II).

Dopamine concentrations were similar among the tested birds until 9 weeks of age at which time saline control birds experienced a spike of central DA, while 8-OH-DPAT birds experienced a lower spike and 5-HT-treated birds continued to decline in central DA concentration ($P < 0.05$; Table II). However, at 18 weeks of age 5-HT-treated birds showed increased central DA levels. Serotonin-treated birds also showed an increase in HVA levels (a dopamine metabolite) at 15 days of age ($P < 0.05$; Table II). Saline, but not 5-HT or 8-OH-DPAT birds, showed central increase in DOPAC concentration at 9 weeks ($P < 0.05$; Table II). Overall, DA turnover was accelerated in 5-HT-treated birds at 15 days of age ($P < 0.05$; Table II). Neither the central NE nor the central EP concentration was significantly altered by treatment ($P > 0.05$; Table II).

Expression of the 5-HT1A receptor gene was altered by both treatment and age. Birds from 5-HT-treated eggs

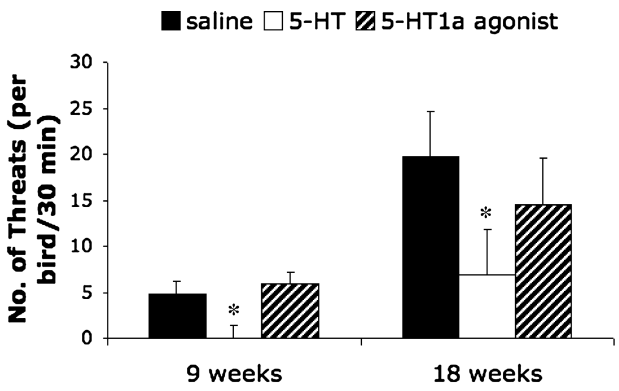

Fig. 1. Effects of prenatal treatment on number of aggressive threats at 9 and 18 weeks of age (±SEM). *Significant difference ($P < 0.05$) between treatment mean and saline control.

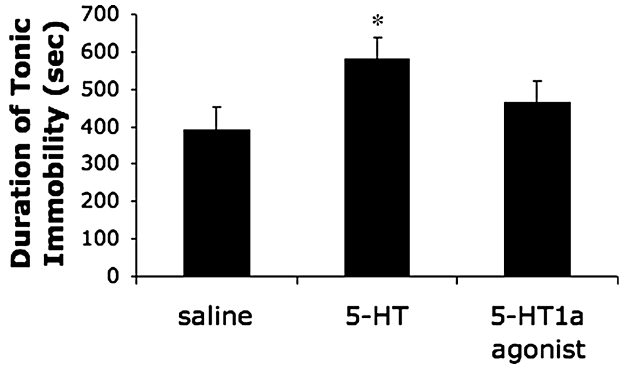

Fig. 2. Effects of prenatal treatment on tonic immobility duration (±SEM). *Significant difference ($P < 0.05$) between treatment mean and saline control.

TABLE II. Central Catecholamine and Metabolite Concentrations for Birds of 5-HT, 5-HT1A Agonist and Saline Treatments at 1 Day, 15 Days, 35 Days, 9 Weeks and 18 Weeks of Age

Treatment	1 day	15 days	35 days	9 week	18 week
NE					
5-HT	50.8 (8.00)	32.0 (7.31)	27.2 (8.96)	40.6 (8.01)	65.5 (8.96)
5-HT1A agonist	68.7 (8.01)	32.5 (6.77)	36.8 (8.96)	53.0 (7.30)	48.4 (8.96)
Saline	50.4 (8.00)	42.4 (7.30)	30.3 (8.96)	70.0 (8.00)	61.1 (8.00)
EP					
5-HT	8.9 (1.53)	3.4 (1.40)	2.5 (1.70)	7.2 (1.53)	10.9 (1.71)
5-HT1A agonist	8.4 (1.53)	3.4 (1.30)	4.8 (1.70)	9.6 (1.40)	10.1 (1.71)
Saline	6.1 (1.50)	3.1 (1.40)	3.2 (1.70)	10.7 (1.53)	11.5 (1.53)
DA					
5-HT	21.0 (4.60)	18.0 (4.20)	14.8 (5.14)	10.9 (4.60)*	22.3 (5.14)
5-HT1A agonist	22.0 (4.60)	15.6 (3.63)	8.9 (5.14)	24.4 (4.20)	8.5 (5.14)
Saline	20.6 (4.60)	16.6 (4.20)	11.5 (5.14)	34.4 (4.60)	11.1 (4.60)
DOPAC					
5-HT	1.8 (0.38)	1.9 (0.32)	1.3 (0.42)	1.2 (0.38)*	1.7 (0.42)
5-HT1A agonist	2.4 (0.38)	1.5 (0.30)	1.4 (0.42)	2.3 (0.34)	1.2 (0.42)
Saline	1.8 (0.38)	1.7 (0.38)	1.5 (0.42)	3.5 (0.38)	2.2 (0.38)
HVA					
5-HT	7.4 (2.68)	17.5 (2.44)*	2.2 (2.99)	4.9 (2.68)	8.4 (2.99)
5-HT1A agonist	10.0 (2.70)	5.1 (2.26)	2.2 (2.99)	9.1 (2.44)	4.7 (2.99)
Saline	5.4 (2.68)	9.9 (2.68)	2.7 (3.45)	11.7 (2.68)	6.6 (2.68)
5-HT					
5-HT	77.6 (11.27)	54.0 (9.52)	53.3 (12.60)	70.7 (11.27)	125.3 (12.60)*
5-HT1A agonist	43.0 (11.27)	51.5 (8.90)	59.0 (12.60)	88.7 (10.30)	72.2 (12.60)
Saline	54.6 (11.27)	55.3 (10.28)	52.8 (12.60)	81.1 (11.27)	51.5 (11.27)
5-HIAA					
5-HT	15.4 (2.48)	10.8 (2.26)	9.1 (2.77)	12.2 (2.48)*	13.7 (2.77)
5-HT1A agonist	21.4 (2.48)	10.3 (1.96)	12.2 (2.77)	14.0 (2.26)*	11.0 (2.77)
Saline	13.9 (2.50)	14.0 (2.50)	10.9 (3.20)	22.5 (2.50)	11.5 (2.50)
DA turn over					
5-HT	0.44 (0.133)	1.08 (0.164)*	0.23 (0.133)	0.57 (0.133)	0.45 (0.133)
5-HT1A agonist	0.56 (0.134)	0.42 (0.176)	0.40 (0.133)	0.47 (0.133)	0.69 (0.133)
Saline	0.35 (0.133)	0.70 (0.182)	0.36 (0.151)	0.44 (0.133)	0.78 (0.133)
5-HT turn over					
5-HT	0.20 (0.070)	0.20 (0.064)	0.18 (0.079)	0.18 (0.070)*	0.14 (0.079)
5-HT1A agonist	0.54 (0.070)	0.21 (0.056)	0.21 (0.079)	0.23 (0.064)*	0.15 (0.079)
Saline	0.35 (0.070)	0.26 (0.070)	0.20 (0.091)	0.43 (0.070)	0.24 (0.070)

*Significantly different from saline (control) treatment ($P < 0.05$).

expressed high levels of 5-HT1A receptor mRNA at day 1 post hatch, and decreased in expression to 18 weeks of age. Alternately, birds from 8-OH-DPAT-treated eggs expressed relatively less 5-HT1A receptor mRNA compared with controls at day 1 of age but increased over time (Fig. 3). Expression of 5HT-1B receptor changed with age ($P < 0.05$), with a relatively low expression at 35 days of age, but was not affected by treatment ($P > 0.05$; Fig. 4).

FA of the shank length and width were elevated in birds from 5-HT but not 8-OH-DPAT-treated eggs ($P < 0.05$; Fig. 5).

DISCUSSION

Serotonin is known to play a role in neural development in addition to and prior to its role as a neurotransmitter [Sodhi and Sanders-Bush, 2004]. As a neurotransmitter, 5-HT is known to play a major role in mood regulation, aggression, and other social and non-social behaviors [Apter et al., 1990; Young and Leyton, 2002]. Disruption in the 5-HT system during development alters neural morphology and later behaviors [Ahmad and Zemenhof, 1978; Lauder, 1990; Lauder et al., 1981]. The type and extent of behavioral changes due to embryonic serotonergic alterations are not fully understood. In mammalian models, increases in 5-HT to the embryo can be met with maternal biochemical feedback. However, in the avian embryo model maternal feedback is excluded. Here, we have shown that a single dose increase in serotonin on day 0 of embryonic development can have lasting effects on birds' behavior and physiology. Similar to the current findings, a single treatment of newborn rats with serotonin causes a life-

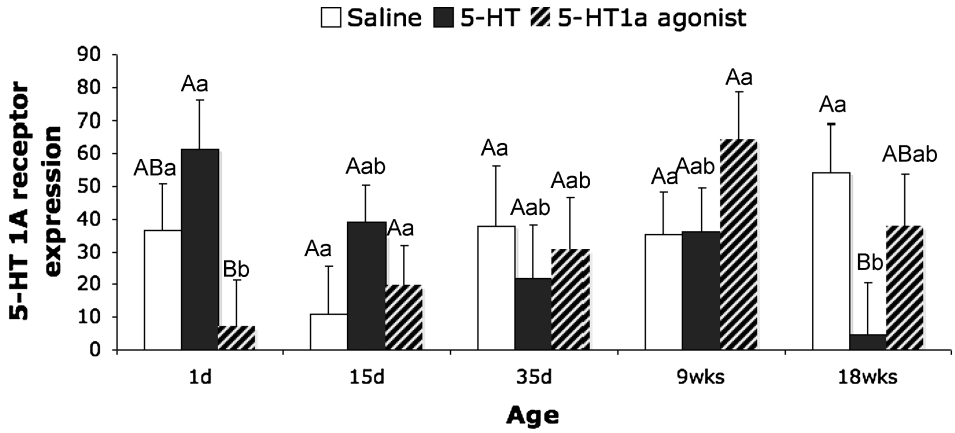

Fig. 3. Effects of prenatal treatment on central serotonin 1B receptor gene expression (±SEM) at different ages. AB, different letters signify differences between treatments and ab, different letters signify differences within a treatment between ages.

long increase in 5-HT concentrations [Csaba et al., 2002]. In conjunction with altered neurotransmission, we have shown a long-term reduction in aggressive behaviors in the chicken animal model.

Serotonin is evident in the embryo prior to the development of biosynthesis processes [Sodhi and Sanders-Bush, 2004]. The initial concentration of 5-HT delivered to the embryo that is used in early neuronal development is provided by the maternal system [Cote et al., 2007]. The amount of 5-HT provided can be affected by maternal genetics or external factors including external stressors and the use of 5-HT altering antidepressants such as SSRIs [Cote et al., 2007; Lattimore et al., 2005]. A single dose increase of 10 μg per egg is shown to alter neural function of both the 5-HT and DA systems here. Reduced aggressiveness in birds seen at 9 and 18 weeks post-treatment was accompanied by a reduction in DA concentrations and an increase in 5-HT, respectively. Antagonism of the DA system and reduced central concentrations of DA have been shown to reduce aggressiveness in birds and mammals [Dennis et al., 2006; Miczek et al., 2002].

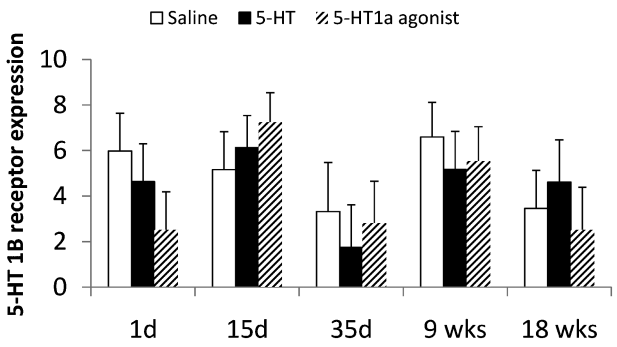

Fig. 4. Effects of prenatal treatment on central serotonin 1B receptor gene expression (±SEM).

Similarly, increased 5-HT levels and agonsim of the 5-HT system also reduce aggressiveness [Dennis et al., 2008; Nelson and Trainor, 2007]. Addition of excess 5-HT prior to incubation leads to a plasticity of both 5-HT and DA systems that is ideal for reduced aggressive behaviors during adolescence and sexual maturity.

Reduced juvenile and adult aggression achieved through increased embryonic 5-HT is accompanied by neurophysiological changes. Serotonin 1A receptor expression was initially elevated but decreased over time. A reduction over time of 5-HT1A receptor expression suggests a difference in pathway plasticity altering serotonin-related behaviors. The absence of 5-HT1A receptors in the prefrontal cortex of mice has been shown to alter DA release as well [Castane et al., 2008]. Interactions between 5-HT and DA can be altered by genetic or epigenetic differences [Couppis and Kennedy, 2008; Nelson and Trainor, 2007]. Epigenetic regulation of these systems can be altered by embryonic treatment. Reduction in 5-HT1A receptor can optimize both the DA and 5-HT systems to cope with social situations without aggressive behavior response.

Antagonism of the 5-HT1A receptor has been shown to reduce aggressiveness in adult animals [Nelson and Trainor, 2007]. Serotonin receptors, including the 5-HT1A receptor, develop as early as the blastula stage [Azmitia, 2010]. However, the current study showed that the application of a 5-HT1A receptor agonist at day 0 of incubation had little effect on adult aggression or fear-related behaviors. Serotonin 1A autoreceptors can function in a negative feedback capacity, pharmaceutical agonism of these receptors can inhibit 5-HT cell firing [Barnes and Sharp, 1999]. These birds exhibited an aggressive behavioral response intermediate between birds from saline and 5-HT-treated embryos. In addition, they exhibit long-term alterations to both the 5-HT and

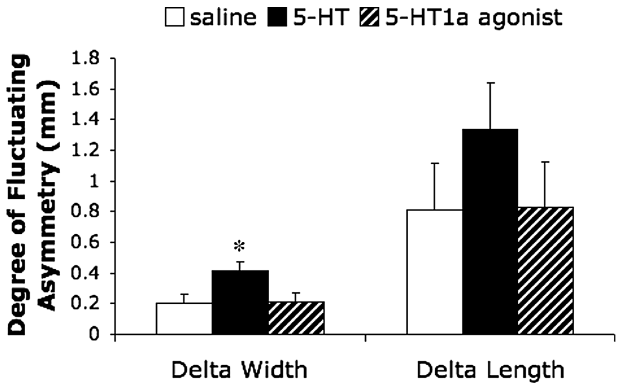

Fig. 5. Effects of prenatal treatment on fluctuating asymmetry of the shank width and length (±SEM). *Significant difference ($P < 0.05$) between treatment mean and saline control.

DA systems. However, these differences are often not as large as the difference between birds from 5-HT-treated embryos and saline controls. This suggests a role for 5-HT1A receptor activation in the behavioral and physiological effects of 5-HT embryonic treatment and the interaction with the DA system. However, to achieve the extent and type of changes seen from treated embryos with 5-HT alone, there must be additional neural targets.

Previous studies have shown that in the developing chick embryo, 5-HT can function as a growth promoting or regulating factor [Ahmad and Zemenhof, 1978]. Serotonin 1A receptor activation can promote dendrite growth [Whitaker-Azmitia, 2001]. Receptor agonists are used to treat dendrite loss; one potential mechanism for this is through S-100 beta proteins that are released through a number of factors including 5-HT1A receptor activation [Whitaker-Azmitia, 2001]. Increased dendrite growth and receptor plasticity can be promoted by 5-HT as a neurogenic compound outside of its role as a neurotransmitter. Serotonin has also been shown to have a strong relationship with brain derived neurotrophic factor (BDNF), two distinctly different systems that can interact or work synergistically to alter development, neurogenesis and plasticity [Martinowich and Lu, 2008]. Serotonin possesses a role in regulating BDNF expression and signaling; this role can greatly alter neural outgrowth and synaptogenesis. The role of 5-HT and neural growth was not specifically investigated in this study; however, our results suggest the need for further work into the effects of serotonin-induced neural growth on behavioral effects in birds.

Reduced aggression was also accompanied by an increase in fear- or anxiety-related behaviors as seen in an increased TI response. This is in agreement with studies that have shown juveniles born of mothers taking SSRI antidepressants (serotonergic agonists) exhibit increased internalizing behavioral problems including anxiety and depression [Oberlander et al., 2009, 2010]. In addition, the alteration to the serotonergic system was not specific to the neural system as seen in an increased fluctuating asymmetry in the shank length and width. This is in agreement with a previous study which found that boys with a higher degree of asymmetry were also less aggressive [Manning and Wood, 1998]. Increased asymmetry in symmetric traits such as legs is an indicator of developmental instability [Dongen, 2006], suggesting that the effect of serotonin altering pharmaceuticals on the development of peripheral systems should be further examined.

The present study provides evidence that a single dose of 5-HT provided in early incubation can have lasting effects on neurophysiology and behavior, reducing aggressiveness through sexual maturity. Neurophysiological effects include alterations of the 5-HT1A receptor system; however, agonism of this system alone will not achieve the same physiological or behavioral changes of increasing overall serotonin. Our results suggest the potential for long-term physiological, behavioral, and social effects on subjects who have been exposed prenatally to alterations in 5-HT including pharmaceuticals agents such as SSRIs.

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